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Effective Bioremediation of Zinc(II) with *Fusarium* sp. in Batch and Continuous Studies

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Designing desirable approach for batch and continuous methods of activity for profoundly effective removal of zinc(II) is of practical importance, given the steady, cost-effective, environmentally friendly attribute. This article offers an answer for foster a continuous stirred tank bioreactor (CSTR) containing single- and two-phase reactors for the viable continuous removal of zinc(II) from aqueous solution by making use of *Fusarium* sp. collected from soil under growing conditions. The different development stages *Fusarium* sp. were tried, including initial metal concentration, pH and growth rate in batch bioreactors. The maximum removal of zinc(II) in the batch studies indicated 63.9 mg/g, at controlled pH: 5.0; and at initial 500 mg/L zinc(II) concentration, which is almost of comparable quantity to the most extreme removal of zinc(II) (52.8 mg/g) obtained in the continuous mode of operation. A continuous flow system operated in staged manner was found to be the ideal strategy for obtaining almost complete removal at lower concentration focus up to 100 mg/L in a single stage bioreactor and a multistage reactor at higher zinc(II) concentrations.

Keywords: Zinc(II), *Fusarium* sp., Metal uptake, Continuous flow system, Continuous stirred tank bioreactor.

INTRODUCTION

In today's world water is such an importance source that life without it is just impossible. With the age of rapid industrialization and urbanization as well as overpopulation, water scarcity is slowly becoming a major problem. Further, water contamination, specifically due to heavy metal being released in industrial wastewater, is a threat to all kinds of living organisms. One of the heavy metals is zinc, which has experienced a special attention in the water industry. Zinc ions can result in anemia, which have a strong affinity for replacing iron in plasma proteins and red blood cells, when bioaccumulated in the food supply chain poses a threat to human life. According to the World Health Organization [1] zinc concentrations in drinking water should not exceed 5.0 mg/L. Zinc(II) ions must be removed or their concentrations must be decreased to the permitted levels before discharging to prevent detrimental effects to the ecosystem [2].

Many industrial processes generate zinc containing wastewaters, including mining and metals, paper, wood preservation, cosmetics, automotive, construction, galvanization, paints and pigments, *etc*. [3,4]. Numerous conventional methods used to remove zinc(II) from aqueous solution include ion exchanger, chemical precipitation, electrolysis, coagulation, membrane filtration [5-7]. In most of these processes, cost and waste generation are factors to consider, especially for low zinc concentrations such as 1-100 mg/L and hence these methods become inefficient or expensive. This is a significant environmental issue because zinc(II) will persist in the environment indefinitely, making remediation difficult. Its persistent nature causes it to accumulate as a result of the food chain, where it finally reaches dangerously high levels in living organisms, posing serious health risks [8]. As a result, environmentally friendly processes must be developed in order to clean the without generating hazardous waste byproducts. Bioremediation was defined as the active or passive capture species, both organic and inorganic from aqueous solutions by microbial biomass, allowing for the environmentally friendly removal (and even further recovery) of these pollutants from the metal polluted effluents.

Living, resting and dead cells are all types of cells of microbial biomass are described to separate zinc(II) from water based solutions [9]. Although, primarily the work to remove

zinc(II) has done with dead fungal cells [10-12] and there is hardly any data on the use of living and not actively growing cells. Dead cells have superiority compared to resting as well as growing cells because they are free of toxicity concerns as well as growth media and nutrient requirements. Furthermore, metal that has been adsorbed can be readily remove from the surface of the microbial biomass and biomass that has been regenerated can be used again. Nonetheless, the most significant drawback is that bioenergetic activity of dead biomass come to a halt as the biomass dry, whereas both living and not actively growing biomass as it may be kept active metabolically. Growing systems, on the other hand, possess a distinct benefit over non-actively growing systems and dead system in which metal has been taken away simultaneously while the cells are growing. Cultivation, harvesting, drying, processing and stockpiling of separate biomass industrial processes, can all be avoided.

However, one of the considerable drawbacks with growing biomass cell systems for metal removal is that biochemical reaction mixture containing growth components is required for organism development and biomass development is reserved when metal concentrations are elevated. This difficulty might be solved using metal-resistant organisms. The tolerance capacities along with uptakes are the critical features of growing cell system considered in a metal biosorption activity.

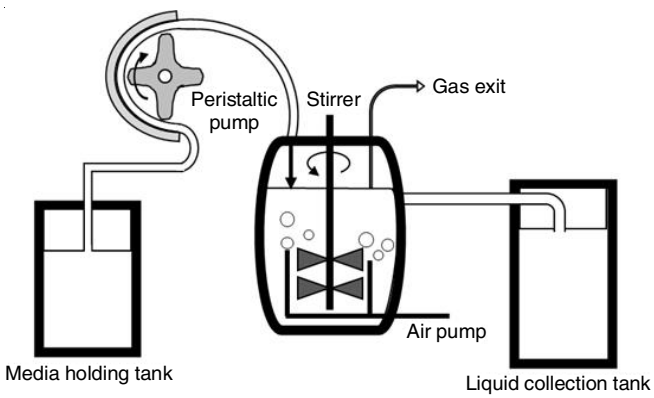
The current work, focused to examine, the highest removal of zinc(II) from the bioreaction mixture with *Fusarium* sp. while it was growing in a batch bioreactor. In a continuous stirred tank bioreactor (CSTR), continuous removal of zinc(II) was looked into with the intention of keeping the microbial cell in a bioactive condition hence, the reactor able to handle considerable amounts of wastewater within a short period of time in comparison to a batch system, where the system can be carrying on with extensive periods of time.

EXPERIMENTAL

Preparation of culture inoculum: At 30 ºC, in a rotary orbital shaker, *Fusarium* sp. was grown in a Erlenmeyer flask (250 mL) and 180 rpm and for this experiment, we used media which contained the ingredients (g/L): $MgSO_4$: 0.1; K_2HPO_4: 0.5; glucose: 10.0; NH_4NO_3: 0.5; NaCl: 1.0; yeast extract: 5.0. The media had a pH: 6.0. For zinc(II) removal studies, an inoculum of 10% (volume/volume) of 36 h matured biomass was used throughout.

Batch studies: Batch test was completed out in a 250 mL bioreaction mixture flask with zinc(II) and of 100 mL media and an inoculum concentration (10%, v/v). The bioreaction mixture vessel was kept at 30 ºC incubation temperature with 180 revolutions per minute. Sulphuric acid was used to adjust the pH to 5.0. The process was tracked over time until it reached the glucose restricting environment. The bioreaction mixture were taken away at regular intervals and processed at 5000 rpm for the duration of about 30 min, after which the liquid from the residual set apart and the remaining zinc(II) and residual sugar levels were found to be determined. The centrifuged biomass was washed, dried as well as the dried quantity of the biomass obtained measured and calculated by weight. The remaining zinc(II) concentration in the sample estimated by atomic absorption spectrophotometer at 214.7 nm.

Studies in continuous stirred tank bioreactor (CSTR): Zinc(II) was removed in a CSTR with a maximum working volume of 3 L. **Scheme-I** shows a CSTR assembly diagram of the bioreaction mixture. This is made up of a column glass bioreactor attached with a media storage tank at the entrance treated solution collection tank at the way out *via* a peristaltic pump. A temperature control module is attached to a heating tape which could be used to heat the reactor from the outside. A multiphase sequential injection system was installed at the rear end of the bioreactor to provide aeration. A magnetic stirrer was installed to get proper mix-up. All the required equipments before use were autoclaved and sterilized air aeration was allowed to pass through an air filter to the bioreaction tank. The bioreactor was first used in the mode of batch studies, with 3 L of broth with zinc(II) and inoculum components, agitated and aerated at 30 ºC. The media with a 10% (v/v) inoculum and at pH 5.0 was used throughout to ensure that a huge number of bioactive cells were obtained (*Fusarium* sp.). After that, process run in continuous studies, with the bioreaction mixture having a calculated dilution rate being fed to the bioreactor *via* the mechanical peristaltic pump. For 20 days, the continuous process was monitored under the conditions of steady state. The liquid sample was collected on a regular basis and the residual zinc(II) and remaining sugar concentrations were determined. The centrifuged biomass was washed, dried, as well as the dried quantity of the biomass obtained measured and calculated by weight. To find out, the best dilution rate for highest removal of zinc(II) at a given concentration of zinc(II) under the optimized equlibrium conditions in a one stage bioreactor, a systematic continuous studies was done with changing dilution rates from 0.01 h^{-1} to 0.04 h^{-1} and varying initial zinc(II) concentration ranging from 50 to 500 mg/L. To achieve complete removal of zinc(II) at further higher concentrations (above 100 mg/L) the continuous studies were also carried out in a two-stage bioreactor at pH 5.0 and dilution rate 0.01 h^{-1} with 500 mg/L zinc(II) initial concentration. The bioreaction mixture having residual zinc(II) concentration in the first stage bioreactor filtered (mesh 300) continuously under steady state conditions. To have huge amount of bioenergetic grown cells, second stage bioreactor initially run-in batch studies (*Fusarium* sp.). The media components were mixed with the filtrate obtained from



Scheme-I: CSTR assembly diagram for bioreaction mixture

the first stage reactor, which contained residual zinc(II) concentration and supply to the connected bioreactor (second stage *via* a mechanical peristaltic pump. Concentrations of residual zinc(II) and residual substrate in the liquids obtained from both the stage reactors (first and second) were determined and the biomass quantity obtained calculated by weight.

RESULTS AND DISCUSSION

Batch studies: The changes in residual glucose concentration (g/L) in the bioreaction mixture along with biomass concentration (g/L) of *Fusarium* sp. with time at various initial zinc(II) concentrations (0-500 mg/L) and at pH 5.0 is depicted in Fig. 1. In the control (absence of metal concentration), the biological growth lag period was measured to be 3 h, which was then observed to be exponentially growing up to 30 h. The growing *Fusarium* sp. was seen within 36 h to have a constant stationary phase. It also shows complete usage of glucose in 36 h in the control (absence of zinc). The biological growth lag period was increasing marginally till 500 mg/L initial zinc(II) concentration. The growth rate of *Fusarium* sp. and the glucose usage with time was observed to be lowered with increasing initial zinc(II) concentration from 0 to 500 mg/L. Although glucose observed to be used entirely in every case. The lowered growth with increasing initial zinc(II) concentration from 0 to 150 µmol/L was also observed with *Arthrobacter* sp. [13]. A significant lowered value in biomass concentration (g/L) appeared with *Arthrobacter* sp. as compared with only 5% decrease observed in the current analysis carried out with *Fusarium* sp. when initial zinc(II) concentration raised from 0 to 500 mg/L. This outcomes evidently show that *Fusarium* sp. identified by the current author can endure higher concentration of zinc(II) in comparison to *Arthrobacter* sp. JM018 observed in the reported studies [13].

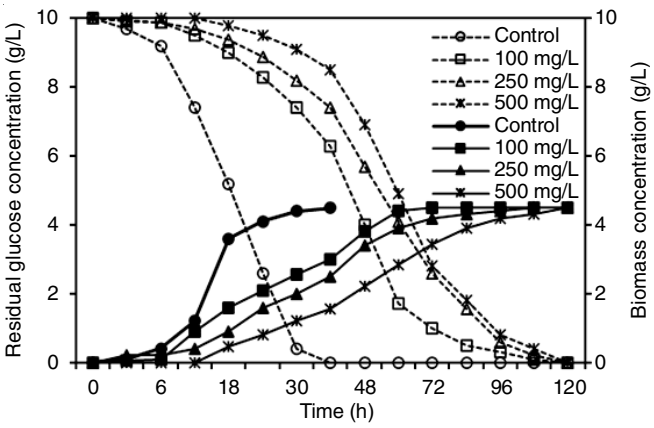


Fig. 1. Changes in biomass concentration and remaining glucose concentration of *Fusarium* sp. over time at various initial zinc(II) concentrations (0-500 mg/L)

The ensuing growth equation was used to determine the specific growth rate (µ) of *Fusarium* sp.

$$\mu = \frac{1}{x}\frac{dx}{dt} \quad (1)$$

Integrated form of the above equation is shown below:

$$\ln\frac{x}{x_i} = \mu(t_x - t_{xi}) \quad (2)$$

where x_i represents the biomass concentrations (g/L) at the starting phase (t_{xi}) and x represents at the exponential phase (t_x), sequentially.

The specific growth rates (µ) and specific zinc(II) removal (mg/g) of *Fusarium* sp. at varying initial zinc(II) concentrations ranging from control (0 mg/L) to 500 mg/L in batch studies is shown in Fig. 2. When the zinc(II) concentration was increasing from control (0 mg/L) to a value of 500 mg/L, the µ value was found to get lower down from 0.06 to 0.02 h^{-1}. It also indicates that specific zinc(II) removal (mg/g) of zinc(II) by *Fusarium* sp. at pH 5.0 and at different initial zinc(II) concentrations from 0 to 500 mg/L shows increasing removal. The specific zinc(II) removal increased from 18.17 (at 100 mg/L) to 63.9 mg/g (at 500 mg/L) with increasing the concentration could be due to accessibility of increased zinc(II) for bioremediation by *Fusarium* sp.

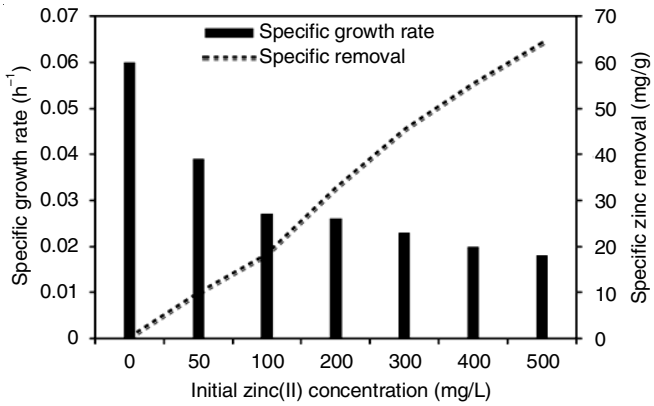


Fig. 2. Specific growth rates and specific zinc(II) removal (mg/g) of *Fusarium* sp. at varying initial zinc(II) concentrations (0-500 mg/L) in batch studies

Continuous studies: For the removal of zinc(II) from the wastewaters in a continuous manner, the experiment was conducted in a CSTR consisting of 3 L apparent volume. Different amount of zinc(II) ranging from 50 to 500 mg/L, with varying dilution rates of 0.01 h^{-1} to 0.04 h^{-1} were used at fixed pH 5.0. Throughout the reactor's transient (temporary phase) process, instantly addition of the uninterrupted media, the remaining zinc(II) amount at first goes higher and the process went on till the bioreactor was stable and reached a phase of equilibrium. The beginning of stable state functioning was observed because of the steady value of remaining zinc(II) obtained in the media. At varying stating zinc(II) concentrations, dilution rate was maintained less than the specific growth rates (µ) of *Fusarium* sp. to keep away from the situation of washout. A test was also carried out with 50 mg/L initial zinc(II) while keeping the dilution rate constant more than the specific growth rate to research zinc(II) transfer under the conditions of wash out.

Table-1 presents the change in residual zinc(II) concentration (mg/L) with time at dilution rates of 0.02, 0.03 and 0.04 h^{-1} and at three different 50, 100 and 200 mg/L metal ion

TABLE-1
CHANGE IN RESIDUAL ZINC(II) CONCENTRATION (mg/L) OVER TIME AT VARIOUS DILUTION RATES AT 50, 100 AND 200 mg/L ZINC(II) CONCENTRATION

Initial Zn(II) concentration (mg/L)	Dilution rate (h^{-1})	Residual zinc(II) concentration (mg/L)										
	Time (h) →	0	1	2	3	4	5	6	7	8	9	10
50	0.04	35	36	37	40	40	41	42	42	42	42	42
	0.03	35	38	40	40	40	40	40	31	28	21	18
	0.02	35	38	40	42	42	40	32	31	20	18	16
100	0.03	68	70	72	75	79	82	86	89	92	93	94
	0.02	68	69	70	72	75	76	79	81	82	85	87
	0.01	68	72	75	76	79	82	85	85	87	87	90
200	0.02	126	200	200	200	200	200	200	200	200	200	200
	0.01	126	148	131	90	76	76	76	76	76	76	76
	Time (h) →	15	20	25	30	35	40	45	50	55	60	
50	0.04	44	46	47	48	48	48	48	48	48	48	
	0.03	15	14	14	14	14	14	14	14	14	14	
	0.02	14	13	12	9	9	9	9	9	9	9	
100	0.03	97	100	100	100	100	100	100	100	100	100	
	0.02	81	79	78	76	76	76	76	76	76	76	
	0.01	88	76	61	49	36	28	24	24	24	24	
200	0.02	200	200	200	200	200	200	200	200	200	200	
	0.01	76	76	76	76	76	76	76	76	76	76	

concentration. At lower initial zinc(II) amount 50 mg/L, under steady-state conditions, the residual zinc(II) concentration lowered with the lowered value of dilution rate. At 50 mg/L initial zinc(II) concentration, at higher dilution rate *i.e.* 0.04 h^{-1}, which is more than the μ value of (0.038 h^{-1}) *Fusarium* sp. there was no significant zinc(II) removal, which clearly indicates the condition of cell washout. The low microbial cell biomass amount (0.5 g/L) of *Fusarium* sp. observed during steady state environment is also supported by this observation. At this stage, remaining glucose assessment also specify partial usage of glucose. At 0.03 h^{-1} dilution rate, zinc(II) uptake was reached upto 36 mg/L, although the glucose was found still unutilized. These suggests that the dilution rate should be reduced even further to achieve total usage of sugar for maximum growth of the microbial cell and improved zinc(II) uptake. However, at 0.02 h^{-1} dilution rate the complete usage of glucose was obtained with a maximum zinc(II) uptake of 41 mg/L. At this dilution rate, as glucose was completely utilized so that zinc(II) uptake process was not continued further with a dilution rate below 0.02 h^{-1}.

Highest removal of zinc(II) removal (76 mg/L) was obtained at concentration of 100 mg/L while keeping the studies running at quite lesser dilution rate of 0.01 h^{-1}. The sugar had been used up completely. At 0.01 h^{-1} dilution rate, at higher zinc(II) concentrations of 200 mg/L, sugar was also observed to be entirely consumed. At the same dilution rate, even at higher zinc(II) concentration of 300 to 500 mg/L, a similar trend of complete usage of sugar was obtained with a maximum zinc(II) uptake of 124-237.6 mg/L (Fig. 3). Dilution rate was kept fixed at 0.01 h^{-1} for rest of all the studies at all the running concentrations because complete usage of sugar was obtained.

Fig. 4 shows the specific zinc(II) uptake (mg/g) at the individual concentration in continuous studies. The diagram clearly shows that the specific zinc(II) removal considered till 500 mg/L, increased with increase in zinc(II) concentration.

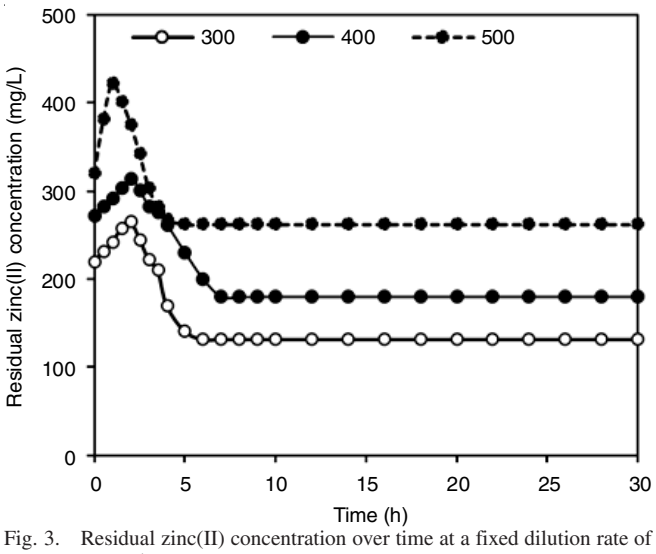


Fig. 3. Residual zinc(II) concentration over time at a fixed dilution rate of 0.01 h^{-1} with 300, 400 and 500 mg/L in continuous studies

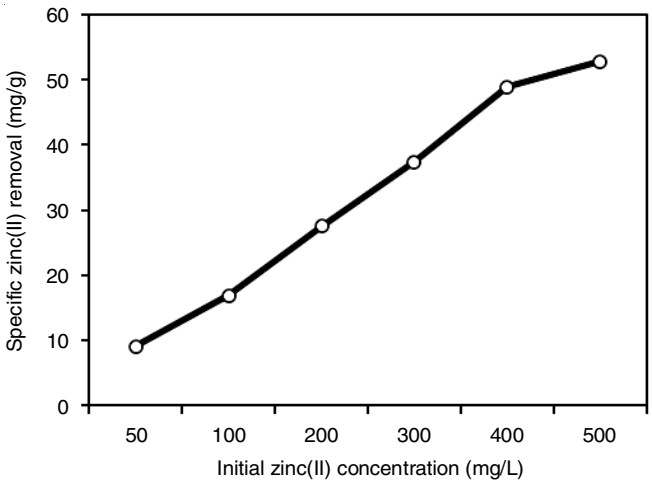


Fig. 4. Specific zinc(II) removal (mg/g) at initial zinc(II) concentrations from 50 to 500 mg/L in continuous studies

A maximum of 52.8 mg/g removal of zinc(II) was observed at 500 mg/L in contrast to 63.9 mg/g found in batch studies. When compared to batch systems, a CSTR will possess the advantage of using a higher volume of wastewaters in a shorter amount of hour and is well appropriate to use in which microbial cells must be kept alive or bioactive and the microbial surroundings must be regulated to a constant condition in order to reach steady growing, that is tough to achieve in batch studies.

Fig. 5 shows the removal (%) of zinc(II) at discrete initial zinc(II) concentrations from 50 to 500 mg/L in batch and continuous studies. A marginal lowering in removal (%) zinc(II) in continuous studies at various initial zinc(II) concentrations (50-500 mg/L) was observed as compared to batch studies. However, at all the concentrations, with increase in initial zinc(II) amount the removal (%) of zinc(II) was decreased. At the concentration of 50 mg/L, the percentage removal was observed as 89%. Whereas, with increase in concentration the removal is decreased. At 500 mg/L, the removal(%) of Zn(II) decreased to 57.5%. This can be explained by the possibility of availability of the binding cell surfaces, which is inhibited due to the existence of increased Zn(II) concentration in the bioreaction mixture.

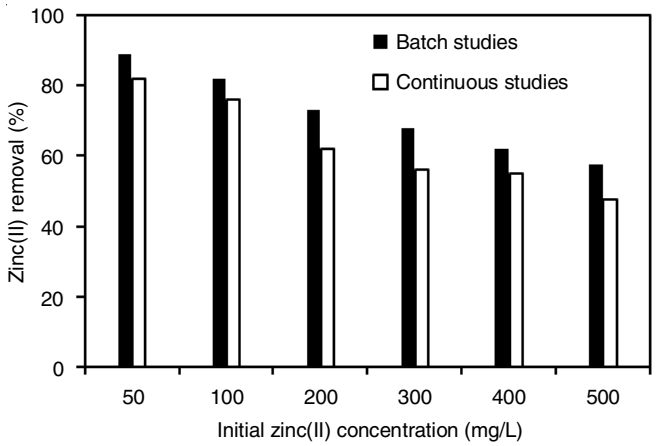


Fig. 5. Zinc(II) removal (%) at various initial zinc(II) concentrations from 50 to 500 mg/L) in batch and continuous studies

When operating under continuous studies, as one stage CSTR didn't have a remarkable impact on zinc(II) removal at higher zinc(II) concentrations despite the fact that glucose was completely consumed. As a result, either raising the glucose amount in the media or operate in a multi-stage bioreactor could improve zinc(II) removal. The higher glucose amount was predicted to rise *Fusarium* sp. microbial cell which ensure the increased zinc(II) uptake. As the microbial cell concentration rises, higher cell surface to surface attachment may reduce the availability of available surface sites, lowering zinc(II) removal. Two-stage operation, as in otherwise, seems further appealing choice for improved zinc(II) removal at larger concentrations. Even though, staged studies will necessitate a greater number of continuous reactors, the sum of glucose and media components required will remain unaltered in a one-step operation and due to the lowered microbial cell concentration of *Fusarium* sp. in every bioreactor, a more effective zinc(II) removal may be possible.

Fig. 6 indicates the changes in residual concentration of zinc(II) over time for a multi(two) stage continuous bioreactor with a dilution rate of 0.01 h^{-1} at a constantly maintained pH 5.0 in the presence of *Fusarium* sp. Under steady state conditions, the total residual zinc(II) concentration in the first stage reactor decreased to 262.4 mg/L from 500 mg/L. From an initial zinc(II) concentration of 262.4 mg/L, the total residual zinc(II) concentration in the second stage reactor decreased to 95 mg/L under stable constant conditions. After two-stage operation, sum of zinc(II) removal was found to be 81% (405 mg/L), compared to 47.52% in one-stage CSTR. The first stage operation resulted in a specific zinc(II) removal of 52.8 mg/g, with additional 37.2 mg/g found in the two-stage operation. At the same initial concentration of zinc, the process continued in multi-staged fashion, *e.g.* with a second-stage bioreactor in continuous process, measured approximately 81% uptake of zinc(II) with *Fusarium* sp. as contrast to 57.5% in batch studies. In comparison to batch studies, continuous studies with staged modes seems found a superior functioning operation because the operation perhaps run considering a longer period with enhanced zinc(II) removal.

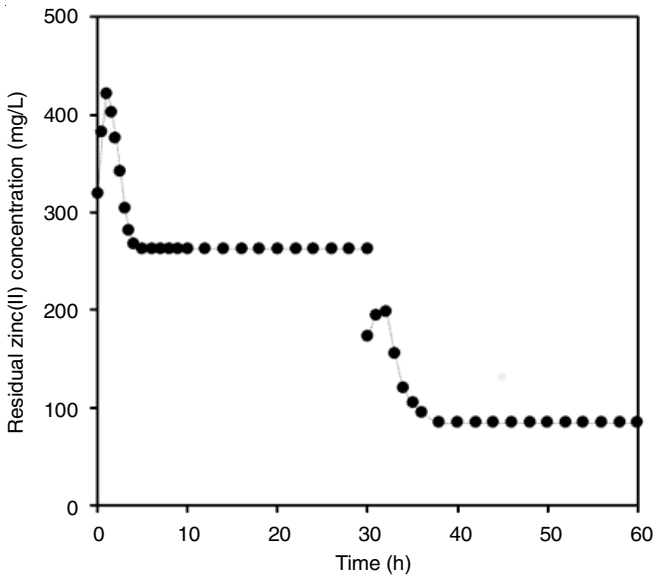


Fig. 6. Residual zinc(II) concentrations over time in a two-stage CSTR at 500 mg/L concentration

Conclusion

A desirable approach for effective removal of zinc(II) from bioreaction mixture using *Fusarium* sp. is of practical importance and involved a complex process of zinc(II) uptake and adsorption by the isolated strain on microbial cell surface. This demands the evolution of worthy working procedure for the effective removal of zinc(II) from wastewaters. In present study, an extremely dominating factor, *i.e.,* residence time takes a significant role in zinc(II) removal with *Fusarium* sp. Although, the removal rate of zinc(II) could be increased by changing the residence time in continuous studies. The limitations enforced on the batch studies can be conquered by running the process of zinc(II) uptake in a CSTR with an ideal dilution rate. The stepped method of working using CSTRs seems more appro-

priate strategical method for improved removal of zinc(II) concentration, when compared to the batch method at a given optimized dilution rate at the same initial zinc(II) concentration.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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